

THAT WHICH IS CLAIMED IS:

1. A method of screening a subject for a glycogen storage disease, comprising the steps of: determining the concentration of hexose tetrasaccharide (Glc)₄ in a biological sample taken from the subject, and comparing the concentration to a reference value, wherein the detection of (Glc)₄ in the biological sample at more than the reference value identifies the subject as affected with a glycogen storage disease.
2. The method of Claim 1, wherein (Glc)₄ has the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.
3. The method of Claim 1, wherein the concentration of (Glc)₄ is determined using a quantitative method.
4. The method of Claim 3, wherein (Glc)₄ is quantified by a method selected from the group consisting of tandem mass spectrometry, mass spectrometry, liquid chromatography, and immunopurification.
5. The method of Claim 1, wherein the concentration of (Glc)₄ is determined using a semi-quantitative method.
6. The method of Claim 1, wherein the glycogen storage disease is selected from the group consisting of Pompe disease (glycogen storage disease type II), glycogen storage disease type III, and glycogen storage disease type VI.
7. The method of Claim 1, wherein the subject is a human subject.

8. The method of Claim 7, wherein the human subject is a neonatal subject.

5 9. The method of Claim 1, wherein the biological sample is a body fluid sample.

10 10. The method of Claim 9, wherein the body fluid sample is selected from the group consisting of blood, plasma, serum, urine, sputum, and amniotic fluid.

11. The method of Claim 10, wherein the body fluid sample is a neonatal blood sample.

15 12. The method of Claim 11, wherein the neonatal blood sample is a dried blood spot.

13. The method of Claim 9, wherein the body fluid sample is a dried urine sample.

20 14. The method of Claim 1, wherein the biological sample is a cell or tissue sample.

25 15. The method of Claim 1, wherein the reference value is a predetermined value.

16. The method of Claim 1, wherein the reference value is based on (Glc)₄ concentrations found in a matched population of subjects.

30 17. The method of Claim 16, wherein the matched population of subjects is an unaffected population of subjects.

18. The method of Claim 1, further comprising the step of performing additional diagnostic testing on a subject that has been identified as affected with a glycogen storage disease.

5 19. A method of screening a subject for Pompe disease (glycogen storage disease type II), comprising the steps of: determining the concentration of hexose tetrasaccharide (Glc₄) in a biological sample taken from the subject, and comparing the concentration to a reference value;

10 wherein the detection of (Glc)₄ in the biological sample at more than the reference value identifies the subject as affected with Pompe Disease.

15 20. The method of Claim 19, wherein (Glc)₄ has the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.

21. The method of Claim 19, wherein the concentration of (Glc)₄ is determined using a quantitative method.

20 22. The method of Claim 20, wherein (Glc)₄ is quantified by tandem mass spectrometry.

25 23. The method of Claim 22, wherein the oligosaccharides in the biological sample are derivatized with butyl-para-aminobenzoic acid prior to quantification by tandem mass spectrometry.

24. The method of Claim 22, wherein the quantification by tandem mass spectrometry is standardized using a [U-¹³C]glucose labeled hexose tetramer as an internal standard.

25. The method of Claim 24, wherein the internal standard comprises a [U-¹³C] labeled hexose tetramer having the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.

5 26. The method of Claim 19, wherein the concentration of (Glc)₄ is determined using a semi-quantitative method.

27. The method of Claim 19, wherein the reference value is a predetermined value.

10 28. The method of Claim 27, wherein the predetermined reference value is based on (Glc)₄ concentrations found in a matched population of subjects.

15 29. The method of Claim 28, wherein the matched population of subjects is an unaffected population of subjects.

20 30. The method of Claim 19, further comprising the step of performing additional diagnostic testing on a subject that has been identified as affected with Pompe disease.

25 31. A method of screening a neonatal subject for Pompe disease (glycogen storage disease type II), comprising the steps of determining the concentration of hexose tetrasaccharide (Glc)₄ in a biological sample taken from the neonatal subject, wherein (Glc)₄ has the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc, and comparing the concentration to a reference value;

30 wherein the detection of (Glc)₄ in the biological sample at more than the reference value identifies the neonatal subject as affected with Pompe Disease.

32. A method of monitoring the clinical condition of a subject with Pompe disease (glycogen storage disease II), comprising the steps of: determining the concentration of hexose tetrasaccharide (Glc)₄ in a biological sample taken from the subject, and comparing the concentration to a reference value;

wherein the detection of (Glc)₄ in the biological sample at more than the reference value is indicative of the clinical condition of the subject.

33. The method of Claim 32, wherein (Glc)₄ has the structure $\alpha\text{-D-Glc}(1\rightarrow6)\text{-}\alpha\text{-D-Glc}(1\rightarrow4)\text{-}\alpha\text{-D-Glc}(1\rightarrow4)\text{-D-Glc}$.

34. The method of Claim 32, wherein the subject is undergoing treatment for Pompe disease.

35. The method of Claim 34, wherein the treatment is selected from the group consisting of enzyme replacement therapy, gene therapy, or dietary therapy.

36. The method of Claim 34, wherein said monitoring is carried out to determine whether to commence or re-initiate treatment of the subject for Pompe disease.

37. A method of assessing the efficacy of a therapeutic regime in a subject with Pompe disease (glycogen storage disease type II), comprising the steps of: determining the concentration of hexose tetrasaccharide (Glc)₄ in a biological sample taken from the subject, and comparing the concentration to a reference value;

wherein the detection of (Glc)₄ in the biological sample at more than the reference value is indicative of the efficacy of the therapeutic regime in the subject.

38. The method of Claim 37, wherein (Glc)₄ has the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.

5 39. A method of screening a neonatal subject for Pompe disease (glycogen storage disease type II), comprising the steps of: determining the concentration of hexose tetrasaccharide (Glc)₄ by tandem mass spectrometry in a dried blood spot from the neonatal subject, and comparing the concentration to a reference value;
10 wherein the detection of (Glc)₄ in the biological sample at more than the reference value identifies the neonatal subject as affected with Pompe Disease.

40. The method of Claim 39, wherein (Glc)₄ has the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.
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41. The method of Claim 39, wherein the quantification by tandem mass spectrometry is standardized using a [U-¹³C]glucose labeled hexose tetramer as an internal standard.
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42. The method of Claim 41, wherein the internal standard comprises a [U-¹³C] glucose labeled hexose tetramer having the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.

25 43. A method of determining the concentration of an oligosaccharide in a biological sample, comprising determining the concentration of hexose tetrasaccharide (Glc)₄ by tandem mass spectrometry in a biological sample taken from a subject.

44. The method of Claim 43, wherein (Glc)₄ has the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.

45. The method of Claim 43, wherein the oligosaccharides in
5 the biological sample are derivatized with butyl para-aminobenzoic acid
prior to quantification by tandem mass spectrometry.

46. The method of Claim 43, wherein the method further
comprises a concentration step prior to said quantifying step.

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47. The method of Claim 46, wherein said concentration step
comprises immunoprecipitation.

48. The method of Claim 43, wherein the biological sample is
15 selected from the group consisting of blood, plasma, serum, urine,
sputum, and amniotic fluid.

49. The method of Claim 43, wherein the biological sample is
selected from the group consisting of blood, plasma, and serum.

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50. The method of Claim 43, wherein the biological sample is
a neonatal blood sample.

51. The method of Claim 43, wherein the biological sample is
25 a neonatal urine sample.

52. The method of Claim 43, further comprising the step of
reducing the concentration of glucose in the biological sample prior to
said quantifying step.

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53. The method of Claim 43, wherein the quantification by tandem mass spectrometry is standardized using a [U-¹³C]glucose labeled hexose tetramer as an internal standard.

- 5 54. The method of Claim 52, wherein the internal standard comprises a [U-¹³C] labeled hexose tetramer having the structure α -D-Glc(1→6)- α -D-Glc(1→4)- α -D-Glc(1→4)-D-Glc.